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L5: Entry 3 of 7 File: USPT Nov 7, 2000

DOCUMENT-IDENTIFIER: US 6143950 A
TITLE: Plant steroid 5.alpha. reductase, DET2

DEPR:
Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the DET2 sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of the amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, et al., Nucl. Acid Res., 9:879, 1981). Alternatively, a subtractive library, as illustrated herein is useful for elimination of non-specific cDNA clones.

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L3: Entry 4 of 34 File: USPT Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214968 B1
TITLE: Fibroblast stimulating growth factor 1 (FsF-1) and the early detection of fibrosis

BSPR:
By "isolated", as used herein in reference to DNA, is meant a DNA that is not immediately contiguous with (i.e., covalently linked to) both of the coding sequences with which it is immediately contiguous in the naturally occurring genome of the organism from which the DNA of the invention is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector (e.g., an autonomously replicating virus or plasmid), or into the genomic DNA of a prokaryote or eukaryote; DNA which exists as a separate molecule independent of other DNA sequences such as a cDNA or genomic DNA fragment produces by chemical means (e.g., polymerase chain reaction, ligase chain reaction), or by restriction endonuclease treatment; and recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence(s). Also included in the isolated DNAs of the invention are single-stranded DNAs which are generally at least 10 nucleotides long, preferably at least 18 nucleotides long, more preferably at least 30 nucleotides long, and ranging up to full length of the gene or CDNA encoding a FsF-1 polypeptide. The single-stranded DNAs can also be detectably labelled for use as hybridization probes, and can be antisense. Preferably, the isolated DNA hybridizes under conditions of high stringency to all or part of the DNA sequence shown in FIG. 18 (SEQ ID NO.: 1), FIG. 25 (SEQ ID NO.; 2) or FIG. 26 (SEQ ID NO.: 3). By "high stringency" is meant, for example, conditions such as those described herein below for the isolation of human FsF-1 cDNA (also see Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989). Most preferably the animal is a mouse or a human, and the DNA sequence encodes substantially all of the amino acid sequence shown in FIG. 18 (SEQ ID NO.: 1), FIG. 25 (SEQ ID NO.: 2) or FIG. 26 (SEQ ID NO.: 3). The DNA of the invention can be incorporated into a vector [which may be provided as a purified preparation (e.g., a vector separated from the mixture of vectors which make up a library)] containing a DNA sequence encoding an FsF-1 polypeptide of the invention or a fragment of the FsF-1 polypeptide, and a cell or essentially homogenous population of cells (e.g. prokaryotic cells, or eukaryotic cells such as mammalian cells) which contain the vector or the isolated DNA described above. By "essentially homogenous" is meant that at least 99% of the cells contain the vector of the invention (or the isolated DNA). Preferably, the vector is capable of directing expression of an FsF-1 polypeptide (for example, in a cell transfected or transformed with the vector).